What is the ecological significance of laboratory temperature selection in anuran Amphibia?

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Temperature selection by a toad (*Bufo viridis*), was studied in the laboratory. This species covers a wide geographical distribution, and is exposed to different temperatures in its natural habitats. A temperature gradient was constructed within a 1.2 m tunnel in the laboratory, and the toads selected a preferred temperature around 27°C, when allowed free choice. Acclimatization to an ambient temperature of 16°C lowered the selected temperature by some 3-4°C. As reported in the literature, many anuran species with different climatic backgrounds share a relatively narrow range of selected preferred temperature in the laboratory. We suggest that the laboratory selected temperature represents an upper set-point that provides anurans with the information to evade high lethal temperatures.

INTRODUCTION

Despite their high vulnerability to ambient conditions, amphibians have invaded many regions of the globe in specific niches. Because water permeability of their moist skin is high, it demands that they avoid permanently hot and dry regions (JØRGENSEN, 1950). Physiological regulatory temperature control in amphibians is poor or absent (BRATTSTROM, 1979), but they use behavioural means to select optimal temperature (HEATH, 1970). Some amphibians that were studied in the field regulate a relatively constant temperature behaviourally (LILLYWHITE, 1970; SINSCH, 1984), while some species that inhabit dry regions burrow (KATZ, 1989, PINDER et al., 1992). In other species, protective mechanisms were developed, such as waxing or cocooning (LOVERIDGE, 1976), that greatly reduce the water permeability of their integument (SHOEMAKER & NAGY, 1977) In either case, in hazardous environments, most amphibians seek shelter and only a few, adaptable species, face the ambient conditions and remain active (SHOEMAKER & NAGY, 1977; KATZ, 1989).

Bufo viridis is one of a few adaptable anurans that can be acclimatized to a wide variety of osmotic and thermal environmental conditions (TERCAFS & SCHOFFENIELS, 1962; KATZ & GABBAY, 1986; KATZ & GIL, 1997). It has a wide area of distribution, extending from temperate south Scandinavia to hot and arid regions in Israel, Egypt and north Africa. *Bufo viridis* is nocturnal and is normally active throughout the year; it burrows, but only intermittently, in conditions of water shortage (Degani et al., 1984; Katz & Gabbay, 1986; Hoffman & Katz, 1994). In this study we investigated the temperature selection of *Bufo viridis* in laboratory conditions, and examined the effects of temperature acclimatization and dehydration on this behaviour.

Materials and methods

Bufo viridis from Israel (more than 20 individuals, belonging to both sexes) were used in this study. Average weight of the toads was 34 g (ranging between 25 and 40 g), and they were kept in the laboratory at room temperature (20-24°C) with free access to tap water. The toads were force-fed ox liver weekly, maintaining constant weight.

Temperature selection of the toads was determined in a temperature gradient that was produced in an opaque plastic tunnel (130 × 14 × 14 cm) heated by two blackened 75 W bulbs, one at each end, and cooled from the outside with dry ice at about a third of the length. Thus, an asymmetric temperature gradient was produced, with a profile that spans over nearly 15°C as shown in fig. 1. Relative humidity in chamber did not exceed 60 %, and the tunnel was dry. The toads were placed singly into the centre of the tunnel, and were allowed 30 min to settle. Changes of position were recorded (through a mirror, to avoid external interference) for several hours, and time spent at various distances from one end were calculated in relation to ambient temperature.

The relationship between body and ambient temperatures was studied separately, in a chamber (10 × 6 × 6 cm) that was kept in a temperature-controlled water bath. The ambient temperature was changed stepwise, by 2-5°C each time, only after the body (and skin) temperature was stabilized (which took no less than 30 min). Temperature was measured with a precision of 0.1°C by a thermistor (YSI, Ohio telethermometer) that was inserted about 2 cm into the cloaca.

Results

Figure 2 shows that, in laboratory conditions, body temperature in *Bufo viridis* (4 specimens) was related almost linearly (in the range 5-35°C) to the ambient temperatures at steady state. The slope of the regression line over the whole range was $y = 0.88\ x - 0.07$; $r^2 = 0.95$ (y is body temperature in degrees centigrade and x is ambient temperature). This relationship deviated at the higher temperatures (range 25-35°C), having a steeper regression line ($y = 1.12\ x - 7.73$; $r^2 = 0.78$).

Toads that were acclimatized at room temperature (ca 22°C), selected a preferred ambient temperature of 27-28°C (fig. 3). The toads spent most of the time in the gentler

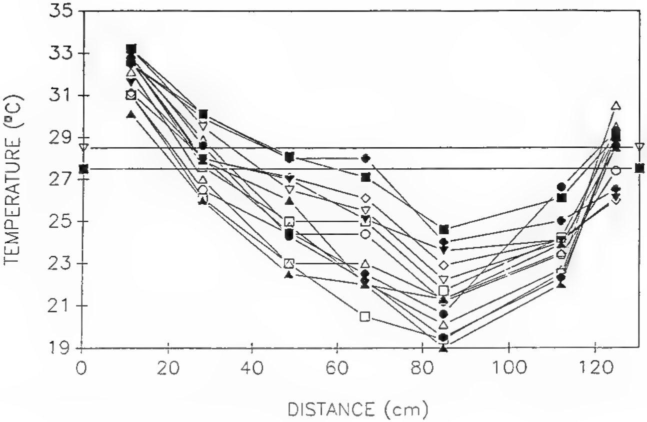

Fig. 1. – Temperature gradients along the (120 cm) tunnel, as recorded in the individual experiments (n = 13). An asymmetric gradient was created by placing two heat sources at each end of the tunnel, and a cooling region located in the third of the distance between them. Note that the toads selected voluntarily a narrow temperature range, indicated by the two horizontal lines.

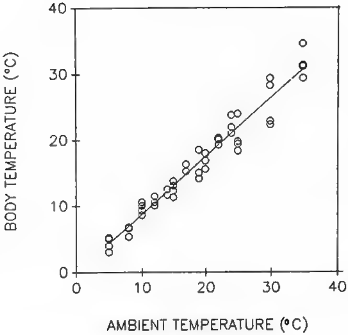

Fig. 2. Relationship between body and ambient temperature in *Bufo viridis*. The regression line equation is y = 0 88 x -0 07, r^2 = 0 95 (5 toads acclimatized at room temperature, ca. 22°C). Weight of the animals was 30-35g.

gradient, with about 20 % of the total time at temperatures below 27°C. As shown in fig. 1, the selected temperature (27-28°C) occupied no more than 15 % of the total temperature profile, including part of the steeper gradient in the tunnel. In control experiments with no temperature gradient, 3 toads that were placed individually in the tunnel explored it over its length and stationed themselves at the ends for most of the time. No other preferred points were observed. This preference disappeared in the presence of the thermal gradient.

Acclimatization of the toads at 16°C for over 3 weeks resulted in a shift in the distribution pattern of the selected temperature (fig. 3), so that now 75 % of total time was spent below 27°C. The difference in the preferred temperature between the two conditions was highly significant ($P > 0.001$; $n = 6$). Fast dehydration (by approximately 15 % gross weight in less than a day) also changed the distribution pattern of preferred temperature, with shorter time spent above 27°C (fig. 4).

DISCUSSION

Our experiments are in accord with previous observations on other species (HUTCHISON & DUPRE, 1992, for a review) and show that when the ectothermic terrestrial anuran *Bufo viridis* is allowed a free choice of temperature in the laboratory, it selects a relatively narrow range of ambient temperatures and spends most of the time at 26-28°C. Preliminary unpublished observations that we made on the African species *Bufo regularis* gave similar results.

Body temperature of amphibians is determined largely by external physical conditions, including ambient temperature, relative humidity (MELLANBY, 1942; MALVIN & WOOD, 1991) and resistance to water loss (SNYDER & HAMMERSON, 1993). Their body temperature is usually somewhat lower than ambient, due to continuous evaporation, although in a number of montane species body temperature is maintained nearly always above ambient (LILLYWHITE, 1970; VALDIVIESO & TAMSITT, 1974; SINSCH, 1989). In natural conditions, amphibians employ mostly behavioural means to regulate their body temperature (LILLYWHITE, 1970); they either bask, as e.g. in *Hyla labialis* (VALDIVIESO & TAMSITT, 1974), or they shelter, to avoid over-heating. They must optimize between conflicting demands of temperature-dependent biochemical reactions and water balance (BRATTSTROM, 1979).

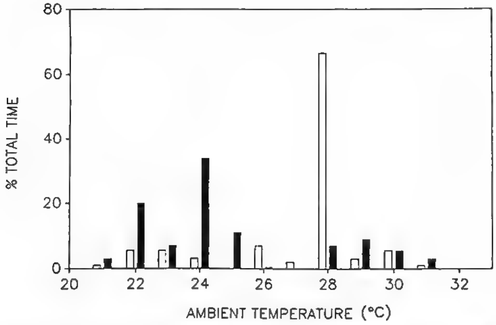

Fig. 3. – Effect of temperature acclimatization on the preferred ambient temperature in *Bufo viridis* Summed results of 6 sessions. Open bars, animals acclimatized at room temperature (ca 22°C); dark bars, animals acclimatized at 16°C.

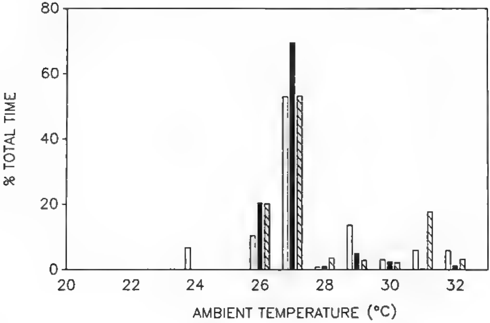

Fig. 4. Effect of dehydration on preferred ambient temperature in *Bufo viridis*. Summed results of 4 individuals on 4 separate occasions in each condition. Open bars, acclimatized at room temperature (ca. 22°C), dark bars, dehydrated by 15 % for 16 h at room temperature, hatched bars, dehydrated, water bath present in the terrarium.

Selected preferred temperature seems to be an inherent property, common to many or most anurans, and does not seem to be related to the species ecological background. *Bufo regularis*, for example, is an African species that cannot survive at temperatures below 8°C (Katz & Gil, 1997), and yet it displayed a similar preferred temperature as did *Bufo viridis*, which survives at near freezing temperature. In many species, the laboratory preferred temperature and the temperature activity in their habitat were found to be quite removed from each other (Clausen, 1973), although in some species these temperatures met with one another, as was found in the aquatic *Rana catesbeiana* (Lillywhite, 1970, 1971). The many studies of laboratory preferred temperature, performed on a large number of amphibians, revealed a narrow range of preferred temperature (between 26 and 30°C) which was selected by more than 75 % of adults among over 40 species of anurans that were tested (Hutchison & Dupre, 1992). Only a few species, including juveniles, chose within the range 16-25°C, while ca. 10 % opted for 31-34°C; this was so, despite the large differences in the experimental methods and acclimatization protocols. This is even more pronounced in the data summarized by Strubing (1954), where only two anurans (*Bombina* spp.) deviated from the 26-33°C range of 34 listed species. Therefore, temperature selection in the laboratory does not reflect the thermal conditions of natural habitats of the animals which vary widely. Rather, it indicates an essential requirement of some fundamental importance to anurans. Some investigators suggested that the amphibian preferred temperature could represent ectothermic equivalence of the endothermic set-point (HUTCHISON & DUPRE, 1992), but this thesis does not seem to be tenable.

If the selected preferred temperature is not related to the thermal background of the animal in nature, what then could it signify? We propose that the laboratory-selected temperature represents a set-point that demarcates a limit to the tolerated temperature. This is supported by the finding that the upper lethal temperature of most toads and frogs – with the notable exceptions of *Phyllomedusa sauvagei* (~40°C) and *Bufo calamita* (~37°C) – is 32-33°C (KIRK & HOGBEN, 1946), much lower than in reptiles, which are also ectothermic. Although amphibians do not possess physiological thermoregulatory mechanisms, they seem to be equipped with a temperature set-point and with cold- and warm-sensitive neurons in the skin (CABANAC & JEDDI, 1971; DUPRE et al., 1986). These are necessary components in any feed-back control system, and provide information that can be used either in physiological temperature regulation or, if not available, for activation of behavioural escape mechanisms. Lacking effective physiological thermoregulatory mechanisms, physical activity and metabolic rate in Amphibia will continue to increase as ambient and body temperatures increase. Therefore, an upper temperature set-point is required to supply safety command to evade lethal temperatures.

In conclusion, we found that *Bufo viridis* and *Bufo regularis* selected a similar preferred temperature in the laboratory, although they show entirely different sensitivity to temperature. Published data and the present study indicate that, in the laboratory, many or most anurans select a preferred temperature from within a relatively narrow range. This temperature is often far removed from, and higher than, the one encountered in the field during their periods of greatest activity. It is suggested that the preferred temperature represents an upper set-point that provides the animals with the information to evade high temperatures.

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